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Bioanalytical assay for the quantification of the ALK inhibitor lorlatinib in mouse plasma using liquid chromatography-tandem mass spectrometry

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ABSTRACT

A bio-analytical assay for the first third generation ALK inhibitor lorlatinib in mouse plasma was developed and validated. Ten-µl plasma samples were prepared by adding rucaparib as the internal standard and precipitation of the plasma proteins. For LC-MS/MS analysis, compounds were eluted at 0.5 mL/min and separated on a 3-µm particle-size, polar embedded octadecyl silica column by gradient elution using 0.1% of formic acid (in water) and methanol. Compounds were monitored with positive electrospray ionization using a triple quadrupole mass spectrometer in selected reaction monitoring mode. The assay was fully validated in the 2–2000 ng/mL calibration range. Within−day (8.0–11.6%) and between−day (10.0–15.0%) precisions and accuracies (99.0–113.3%) were within acceptable range. Plasma samples were deemed stable for 6 h at ambient temperature, during three freeze-thaw cycles and for 2 months at −30 °C. Finally, the new assay was applied successfully to pilot pharmacokinetic studies in male and female wild-type mice.

1. Introduction

Lorlatinib (PF-06463922; Fig. 1) is an orally available ATP-competitive selective inhibitor of receptor tyrosine kinases, the anaplastic lymphoma kinase (ALK) and the related C-ros oncogene 1 (ROS1) and it is the first drug belonging to the third generation of ALK inhibitors [1]. ALK performs a key role in nervous system development and its dysregulation is associated with several tumors; ROS1 plays a prominent role in cell growth and survival of cancer cells. ALK inhibitors are now considered effective agents against ALK positive non-small cell lung cancer (NSCLC) [2]. However, their effectiveness over time tends to decrease due to the emergence of mutations resistant to therapy with crizotinib (first generation) and second generation ALK inhibitors as well [1,2]. The ALK protein, encoded by the *ALK* gene, represents a clinical target in adult cancers in which the ALK domain is fused to an array of amino-terminal partners, such as the echinoderm microtubule-associated protein-like 4 (EML4) in NSCLC or nucleophosmin (NPM) in anaplastic large cell lymphoma (ALCL) [3]. Since EML4-ALK was recognized as an attractive drug target, the development of ALK inhibitors resulted in approval of crizotinib, ceritinib, alectinib, brigatinib, entrectinib and lorlatinib to treat NSCLC patients harboring ALK fusions [3]. Currently, lorlatinib is in phase 1/2 clinical trials for treatment of ALK-driven cancers [4], specifically in patients after failing crizotinib and ceritinib [1]. It was reported that it is highly potent against known clinically acquired *ALK* mutations with superior brain penetration compared to previous generations of ALK inhibitors [1].

In order to support (pre)clinical studies with lorlatinib, it is necessary to develop bio-analytical assays that are able to monitor the

Abbreviations: ALK, anaplastic lymphoma kinase; EMA, European Medicines Agency; EML4, echinoderm microtubule-associated protein-like 4; ESI, electro spray ionization; FDA, Food and Drug Administration; IS, internal standard; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LLOQ, lower limit of quantification; NPM, nucleophosmin; NSCLC, non-small lung cancer; QC, quality control; ROS1, proto-oncogene tyrosine kinase; SRM, selected reaction monitoring

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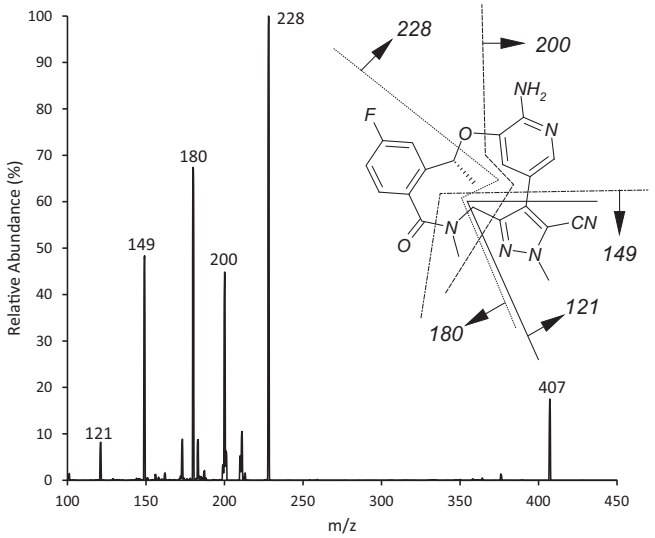

Fig. 1. Chemical structure and product spectrum formed by collision induced dissociation of the protonated molecule of lorlatinib, *m/z* 407.1@ − 25 V.

concentration of this drug in different biological matrices. Plasma concentrations of lorlatinib were previously determined by LC-MS/MS [5,6], using protein precipitation to pretreat the plasma samples. Analytical and validation details given for this assay were, however, very limited. Therefore, in the present study a straightforward bioanalytical assay for lorlatinib in mouse plasma was set up using LC-MS/MS, providing complete analytical details and a full bioanalytical validation for small mouse plasma samples. The present assay also uses a simple protein precipitation step followed by LC-MS/MS quantification and it could be successfully applied for these samples.

2. Materials and methods

2.1. Chemicals and reagents

Lorlatinib (> 99.9%) was obtained from TargetMol (Boston, USA) and rucaparib (phosphate salt, > 98.5%) from Sequoia Research Products (Pangbourne, UK). LC–MS grade water, acetonitrile of HPLC-S gradient grade quality and methanol of HPLC quality and were acquired from Biosolve (Valkenswaard, The Netherlands). Water purified by reversed osmosis on a multi-laboratory scale was applied for all other purposes than preparing chromatographic eluents. Formic acid (analytical grade) was obtained from Merck (Darmstadt, Germany). Lithium-heparinized plasma (pooled from humans and mice) and plasma of six single mice was obtained from Sera Laboratories (Haywards Heath, West Sussex, UK).

2.2. Chromatography and MS/MS method

The Shimadzu (Kyoto, Japan) chromatographic system was build up from a DGU-14A degasser, two LC10-ADvp-μ pumps, a Sil-HTc autosampler and a CTO-10Avp column oven. Prepared samples were injected (5 μL) on a Varian Polaris C18-A (50 × 2.0 mm, 3 μm, Varian, Middelburg, The Netherlands), protected by an Agilent Polaris C18-A Chromsep guard cartridge (10 × 2.0 mm, 3 μm, Agilent, Santa Clara, USA). The auto injector rack was maintained at 4 °C and the column oven at 40 °C. For the binary gradient, 0.1% (v/v) formic acid in water (A) and methanol (B) were mixed to obtain a total flow rate of 0.5 mL/min. After injection, 20% B was increased linearly to 45% in 1.2 min followed by flushing with 100% B for 0.3 min and equilibration of the column at the initial 20% B for 1.5 min until starting the following injection. The eluate was transferred to the electrospray nebulizer from 0.6 until 2.5 min after injection by switching the MS inlet valve. A TSQ Quantum Discovery Max quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) was used for ionization, ion separation and detection. The Thermo Fisher Xcalibur software (Version 2.0.7 SP1) was used for data collection and control of the mass spectrometer. Positive electrospray ionization was optimized by introducing 0.5 mL/min of a mixture of methanol and 0.1% v/v formic acid in water (50/50, v/v) and mixing the solvent mixture with 5 μg/mL lorlatinib at 5 μL/min. Settings with the highest response were used for monitoring both compounds: 4000 V spray voltage; 400 °C capillary temperature; nitrogen gas settings (arbitrary units) were 60 for sheath, 8 for ion sweep and 10 for the auxiliary gas; the skimmer voltage was set off (0 V); the argon collision gas pressure in SRM mode was 1.5 mTorr. The tube lens off-set was 113 V for lorlatinib and 92 V for rucaparib. Lorlatinib was monitored at *m/z* 407.1 → 121, 180.1, 200.1 and 228.1 at respectively − 41, − 23, − 26 and − 23 V collision energies and rucaparib at *m/z* 324 → 293 at − 17 V collision energy, all with 0.1 s dwell times. Both separating quadrupoles were set at *m/z* 0.7 mass resolution.

2.3. Calibration standards and quality control samples

Stock solutions were prepared in methanol at 250,000 ng/mL for calibration and at 500,000 ng/mL lorlatinib solution for quality control (QCs) samples. The highest calibration solution at 2000 ng/mL was made by diluting the 250,000 ng/mL stock solution in blank mouse plasma; it was stored in polypropylene tubes at − 30 °C until further use. Additional calibration solutions were produced by diluting the highest calibration solution to 1000; 200; 100; 20; 10 and 2 ng/mL with blank mouse plasma. The 500,000 ng/mL lorlatinib stock solution was used to produce QC samples at 1500 (high), 75 (medium), 5 (low) and 2 (lower limit of quantitation, LLOQ) ng/ml by serial dilution with blank mouse plasma. QCs were also stored at − 30 °C until further use.

2.4. Sample preparation

10 μL of plasma was pipetted into a 1.5-ml polypropylene (PP) reaction tube. After addition of 20 μL of 200 ng/mL IS solution, 200 ng/ml rucaparib in acetonitrile, proteins were precipitated by vortex mixing vigorously for approximately 5 s. Centrifugation at 12000 × *g* for 5 min at 10 °C resulted in a clear supernatant of which 20 μL was transferred to a 1.5 mL vial with a 250 μL glass micro-insert. The vial was closed after addition of 100 μL water and placed in the autosampler for injection of 5 μL of the final mixture.

2.5. Bioanalytical method validation

International guidelines (EMA [7] and FDA [8]) were used to obtain validation procedures for this bioanalytical assay.

2.5.1. Calibration

All calibration samples were pretreated in duplicate for each daily calibration. The calibration curve was constructed using weighted ($1/x^2$; x is the concentration of analyte) linear regression and data were calculated from the peak area of the analyte relative to the IS.

2.5.2. Precision and accuracy

Analytical performance (within- and between-day) was calculated after six-fold analysis of each QC in three analytical runs on separate days for all four QC samples (total: $n = 18$ per QC). In addition, dilution integrity was tested on one day ($n = 6$) at 4000 ng/ml lorlatinib after 5-fold dilution of 10 μL of mouse plasma with human plasma.

2.5.3. Selectivity

Individual mouse plasma samples ($n = 6$) were investigated for the selectivity of the assay. Each sample was analyzed as double blank (no lorlatinib, no IS), blank (no lorlatinib, with IS) and LLOQ spiked (2 ng/